30 VOLATILES BY HEADSPACE GC

30.1 Summary

30.1.1 An aliquot of specimen is diluted semi-automatically with an internal standard (IS) solution (n-propanol) into a glass vial, sealed, and placed in a heated automatic sampler. The concentration of volatiles in a dilute aqueous biological sample is directly proportional to its concentration in the gas phase or headspace. A portion of the resultant headspace vapor above the liquid is automatically injected into a gas chromatograph (GC) equipped with a flame ionization detector (FID). Volatiles are identified by retention time and their concentrations are calculated by comparison to similarly-treated aqueous calibrators by using peak heights or areas. Whenever possible, volatiles should be confirmed by headspace GCMS.

30.2 Specimen Requirements

30.2.1 50 µL of biological fluid(s), tracheal air or tissue is diluted with 450 µL of internal standard for analysis.

30.3 Reagents And Standards

30.3.1 Reference standards of volatiles or gases

30.3.2 n-propanol

30.4 Calibrators, Controls And Internal Standards

30.4.1 The following is an example of an acceptable procedure for the preparation of calibrators. Not all analytes are amenable to quantitative analysis (e.g. freons, isobutane). Other quantitative dilutions may be acceptable to achieve similar results.

30.4.1.1 10% stock solutions. Prepare individual 10% w/v solutions of the analytes by weighing 10 grams of each into a 100 mL volumetric flask and QS to volume with dH_2O. The 10% w/v solutions are used to prepare the working calibration solutions of 0.10%, 0.30%, 0.60% w/v and the 0.20% control solution.

30.4.1.2 0.10% calibration solution. Pipet 1 mL of each 10% stock solution into a 100 mL volumetric flask and QS to volume with dH_2O.

30.4.1.3 0.30% calibration solution. Pipet 3 mL of each 10% stock solution into a 100 mL volumetric flask and QS to volume with dH_2O.

30.4.1.4 0.60% calibration solution. Pipet 6 mL of each 10% stock solution into a 100 mL volumetric flask and QS to volume with dH_2O.

30.4.1.5 0.20% control solution. Pipet 2 mL of each 10% stock solution into a 100 mL volumetric flask and QS to volume with dH_2O.

30.4.2 0.3 mL/L n-propanol internal standard solution. Pipet 300 mL n-propanol standard into a 1 L volumetric flask and QS to volume with dH_2O.

30.4.3 The following are guidelines for the preparation of qualitative standards for identification of analytes that are in the gas phase at room temperature. Due to their low boiling point and high volatility, numerous compounds of interest do not easily lend themselves to the preparation of calibrators and/or quantitative analysis (freons, isobutene, methane etc).

30.4.3.1 Capture of primary standard. In a fume hood, flush a 20 mL headspace vial with the analyte (usually in the form of a pressurized cylinder or can) for several seconds.

30.4.3.2 While continuing to flush the vial, cap vial with septa and hold down until crimped with aluminum cap.

30.4.3.3 Generally, the stock primary standard is of such high concentration that it must be diluted in order to avoid overloading the GC column and/or detector. Serial dilutions of the primary standard may be made into nitrogen purged headspace vials using gastight syringes to transfer aliquots of the headspace from vial to vial.

30.4.3.4 The appropriate dilutions for an analyte will have to be determined via trial and error. The analyte peak should not be so large as to cause a shift in retention time of the internal standard.

30.4.3.5 The final dilution of the standard should be into a vial containing internal standard so that the relative retention times of the analytes may be determined and used to identify the unknowns in case specimens.

30.5 Apparatus

30.5.1 Gas chromatograph with data system, flame ionization detector and a head space auto sampler

30.5.2 Column. Restek Rtx-BAC 1 or BAC 2 capillary column

30.5.3 Glass 20 mL (23 x 75 mm) headspace vials with Teflon septa and aluminum seals

30.5.4 Vial seal crimper

30.5.5 Test tubes or sample cups

30.5.6 Hamilton Microlab Diluter or equivalent

30.5.7 Gastight Hamilton syringes

30.6 Procedure Case samples are prepared and analyzed in duplicate. Calibrators and controls are analyzed singly.

30.6.1 Pour approximately 0.2 mL of calibrator, control, blood or other specimen into a clean test tube or sample cup (this initial step enables visualization of any clots and prevents possible contamination of the original sample by the internal standard solution). NOTE. If analyzing weighed tissue specimens, place the tissue in the headspace vial and add 4.5 mL internal standard solution.

30.6.2 Place the dilutor delivery tip into the specimen, making sure its tip is below the surface of the sample. Activate the dilutor. At this point, the dilutor draws 0.05 mL of sample into its delivery tube.

30.6.3 Withdraw the tip and wipe it with a Kimwipe/tissue paper.

30.6.4 Direct the delivery tip into the appropriately labeled headspace vial and activate the dilutor. The dilutor will dispense the specimen and 0.450 mL of IS solution into the vial.

30.6.5 Flush the dilutor tube as necessary by activating the dilutor one or more times or rinsing with dH_2O, depending on the viscosity or other nature of the specimen. Dispense washings into a waste beaker.

30.6.6 Stopper the headspace vial with the Teflon seal. Vortex or manually shake the vial for several seconds, and place in the sample rack.

30.6.7 Repeat steps 30.6.1 – 30.6.6 for all calibrators and controls.

30.6.8 Seal all headspace vials by crimping the aluminum rings over the Teflon seals.

30.6.9 Load headspace vials in the headspace auto sampler.

30.7 Headspace Analysis

30.7.1 Gas Chromatograph Operational Parameters. The following conditions are recommended starting parameters. Modify the parameters as necessary to achieve optimum operation. Print out the optimum conditions and save them as attachments to this SOP. For approximate retention times of volatiles, see Restek Application Note #59548 on GC Analysis of Commonly Abused Inhalants in Blood Using Rtx BAC1 and Rtx BAC2 Columns.

- Oven 40ºC Isothermal
- Injector 200ºC
- Detector (FID) 250º
  - Hydrogen flow 35 mL/min
  - Air flow 450 mL/min
  - Make-up flow 22.6 ml/min
  - Make-up gas helium
- Inlet
  - Split
  - Split ratio 0.5:1
  - Split flow 3.2 mL/min
  - Total flow 12.8 mL/min
  - Pressure 18 psi constant pressure mode

30.7.2 Headspace Sampler Operational Parameters. The following conditions are recommended starting parameters. Modify the parameters as necessary to achieve optimum operation. Print out the optimum conditions and save them as attachments to this SOP.

- Sample Oven 70ºC
- Sample Valve 85ºC
- Transfer Line 95ºC
- GC Cycle 10.0 min
- Sample Equilibration 3.0 min
- Vial Pressurization 0.91 min
- Loop Fill 0.20 min
- Loop Equilibration 0.05 min
- Sample Inject 1.00 min
- Oven Stabilization 1.0 min
- Agitation Low
- Extractions 1
- Puncture Mode Single

30.7.3 Start the GC by selecting "Run Sequence" under the Run Control menu. Select "Start" on the headspace display monitor to begin the analysis.

30.8 Calculation

30.8.1 Volatiles are identified based on relative retention times compared to calibrators. Identification is performed by instrument software. Retention times for both analyte and internal standard should be within ± 2% of the retention time obtained from the calibrators.

30.8.2 Concentration is calculated automatically by the software based on linear regression of the calibration curve based on peak area or peak height measurement.

30.8.3 Manual calculations involve measuring peak areas or heights and using the following formula:

$$C_i = \frac{A_i}{A_s} \times \frac{A_{st} \times C_e}{A_e}$$

Where C_i = unknown volatile concentration of the sample
C_e = volatile concentration of the standard
A_i = peak area (or height) corresponding to unknown volatile concentration of the sample
A_e = peak area (or height) of volatile in the standard
A_s = peak area (or height) of the n-propanol internal standard in the sample
A_{st} = peak area (or height) of the n-pronpanol internal standard in the standard

30.8.4 Tissue concentration is calculated as follows:

$$\text{Chromatogram concentration} \times \frac{0.5\ g}{\text{weighed amount}} = \text{volatile tissue concentration \% (w/w)}$$

30.9 Quality Control And Reporting

30.9.5 Reporting.

30.9.5.1 Report the volatile concentration of the lower replicate in % by weight by volume truncated to two decimal places.

30.9.5.2 If at all possible, confirm the identity of the volatile by headspace GCMS.

30.10 References

30.10.1 Restek Application Note #59548. GC Analysis of Commonly Abused Inhalents in Blood Using Rtx BAC1 and Rtx BAC2 Columns.

30.10.2 L. C. Nickolls, "A Modified Cavett Method for the Determination of Alcohol in Body Fluids," Nov. 1960, Analyst, Vol. 85, pp 840-942.

30.10.3 B. Kolb, "Head Space Analysis by Means of the Automated Gas Chromatograph F-40 Mulitfract", Bodenseewerk Perkin-Elmer and Co., Technical Manual #15E.

30.10.4 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

30.10.5 G. Machata, "Determination of Alcohol in Blood by Gas Chromatographic Head Space Analysis," Clin Chem. Newsletter, 4(1972), 29.

30.10.6 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, p. 180.

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